J. Physiol. (1970), **210**, *pp.* 151–164
With 4 text-figures
Printed in Great Britain

PASSAGE OF MACROMOLECULES BETWEEN ALVEOLAR AND INTERSTITIAL SPACES IN FOETAL AND NEWLY VENTILATED LUNGS OF THE LAMB

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(*Received 26 January 1970*)

SUMMARY

1. Experiments were performed on exteriorized foetal lambs to measure transfer of macromolecules (proteins and [^{125}I]PVP) between lung alveolar liquid (A_l) and lung interstitial liquid as represented by lung lymph (L_{ly}). Transfer was measured in the foetus and during a 2 hr period of positive pressure ventilation.

2. In thirteen experiments [^{125}I]PVP was introduced into A_l and, after a control period, the lungs were ventilated for 2 hr. In six other experiments the [^{125}I]PVP was injected intravenously; and in two of these the lungs were ventilated for 2 hr. Measurements were made of protein and of [^{125}I]PVP concentration in plasma and L_{ly} collected at intervals throughout the experiment, as well as in A_l obtained before ventilation and at the end of ventilation after degassing the lung. [^{125}I]PVP in the samples was separated into fractions of different Stokes–Einstein radius by gel filtration using Sephadex G-200.

3. Before ventilation there was evidence of a negligible transfer of protein from L_{ly} to A_l (mean protein $A_l/L_{ly} = 0{\cdot}014$) or of [^{125}I]PVP from A_l to L_{ly} (mean [^{125}I]PVP $L_{ly}/A_l = 0{\cdot}00017$). The effect of ventilation for 2 hr was to produce an increase in both ratios, but by a variable amount (after 2 hr ventilation mean protein $A_l/L_{ly} = 0{\cdot}70 \pm 0{\cdot}08$ (S.E. of mean) and mean [^{125}I]PVP $L_{ly}/A_l = 0{\cdot}48 \pm 0{\cdot}09$). By calculating L_{ly}/A_l ratios for [^{125}I]PVP fractions obtained by gel filtration it was shown that in transfer from A_l to L_{ly} during ventilation molecular sieving could be detected, the degree of which was expressed by means of a Relative Sieving Index (RSI). Significant correlations were found between the minimum surface tension of lung extracts, and two other indicators of lung maturity on the one hand, and the protein and [^{125}I]PVP ratios and RSI on the other; so that the more mature the lamb the less the transfer of macromolecules during ventilation and the greater the degree of molecular sieving.

4. By comparing concentrations of [^{125}I]PVP gel filtration fractions in lymph and plasma, when the [^{125}I]PVP had been placed in A_1, we demonstrated some sieving of molecules (in the range 34–15 Å) in their absorption from interstitial space to plasma.

5. It was concluded that in the lungs of the foetal lamb there is an almost complete barrier to the transfer of macromolecules between alveolar and interstitial spaces; that positive pressure ventilation for 2 hr causes a very variable degree of break-down in this barrier, and that the degree of break-down is usually greater in immature than in mature lungs.

INTRODUCTION

In experiments on foetal and new-born lambs Boyd, Hill, Humphreys, Normand, Reynolds & Strang (1969) showed that macromolecules (protein and polyvinylpyrrolidone-PVP) pass fairly readily from plasma to lung lymph; and from measurements of the transfer rates of different sizes of molecule they concluded that the walls of lung capillaries in foetal lambs could be characterized as containing uniform pores 150 Å in radius with in addition a few much larger leaks. The composition of the liquid contained in the alveoli of the foetal lung differs in a number of respects from lung lymph, which we have taken as representative of lung interstitial fluid (Adamson, Boyd, Platt & Strang, 1969). In particular alveolar liquid has a much lower protein concentration than lung lymph, from which we conclude that the layer of alveolar epithelial cells, which can be seen in electron micrographs of lung separating alveolar and interstitial spaces, is likely to be much less permeable to macromolecules than lung capillary walls.

We did the present experiments to measure the transfer of protein and [^{125}I]PVP across alveolar walls in the foetal state and during the first 2 hr of positive pressure ventilation, when a large proportion of the liquid contained in the foetal lungs is transferred across alveolar walls into the interstitial spaces of the lung and is then drained away in lymph (Humphreys, Normand, Reynolds & Strang, 1967). We were particularly interested in the possibility that macromolecules might enter the air spaces of the lung during the first few hours of ventilation due to some kind of break-down in the barrier between alveoli and interstitial space.

METHODS

Experimental procedure

Anaesthesia, dissection, monitoring of foetus. Experiments were performed on nineteen foetal lambs from eighteen pregnant ewes. Four of these lambs, in which [^{125}I]PVP was given intravenously to the foetus, were used also in the experiments

reported by Boyd *et al.* (1969). An estimate of gestational age (obtained from the tupping date given by the farmer) was available for fifteen lambs. Anaesthesia in the ewe was induced with I.V. thiopentone (10 mg/kg) and maintained in nine experiments with I.V. pentobarbitone and in ten experiments with 1 % chloralose I.V. (3 ml./kg). Having exteriorized the foetal lamb by Caesarean section, cannulae were placed in the trachea, a jugular vein and a carotid artery; and the thoracic duct component of lung lymph was collected after excluding non-pulmonary sources of lymph by ligating the cisterna chyli and ablating cardiac lymph trunks as described in Humphreys *et al.* (1967). At the end of the dissection heparin (800 i.u./kg) was injected I.V. into the foetus. Samples of lung lymph were collected over 5–15 min periods throughout the experiment, and samples of carotid artery blood at intervals. The condition of the foetus was monitored by measurements of arterial B.P., heart rate, P_{a,O_2}, P_{a,CO_2} and pH_a as described by Humphreys *et al.* (1967), with the following results (means and ranges): B.P. (mm Hg) = 51 (44–70); heart rate (per min) = 181 (140–225); P_{a,O_2} (mm Hg) = 30 (20–38); P_{a,CO_2} (mm Hg) = 43 (31–50); $pH_a = 7{\cdot}34$ (7·27–7·44).

[^{125}I]*PVP added to alveolar liquid.* In thirteen lambs as much liquid as possible was gently withdrawn from the lungs through a tracheal cannula into a syringe, its volume measured and 0·3–1·0 ml. of a solution containing [^{125}I]PVP was added (3–10 mg PVP; 40–120 μc). The mean volume of alveolar liquid withdrawn = 13·3 ml./kg body weight ± 6·0 S.D. Having taken a sample, the mixture was re-injected into the lungs through the tracheal cannula and mixed with the residual liquid in the lungs, by withdrawing and injecting four times. At the end of 1 hr as much liquid as possible was again withdrawn by syringe, a sample taken and the liquid returned to the lung. Mechanical ventilation was then started, the umbilical cord being tied 30 min later.

[^{125}I]*PVP given intravenously.* In six lambs (one of 125 days and five of 140–146 days gestation) 5 ml. of a solution containing [^{125}I]PVP in 0·9 % NaCl (10–15 mg PVP; 200–300 μc) was injected through the jugular vein cannula. At the end of a control period lasting 2·0–3·5 hr, during which samples of lymph and carotid arterial blood were taken, as much alveolar liquid as possible was gently withdrawn by syringe through the tracheal cannula, a sample taken and the liquid returned to the lung. In two of these experiments mechanical ventilation was then started.

Positive pressure ventilation. The lungs were ventilated by attaching the tracheal cannula to a volume cycled positive pressure ventilator (Harvard Apparatus Co., Dover, Mass.) set to deliver a tidal volume of 9–11 ml./kg body weight at a frequency of 30/min, and with the end expiratory pressure held at 3–6 cm H_2O.

In nine experiments the peak tracheal pressure during ventilation was recorded by means of a Sanborn pressure transducer. As in previous experiments (Humphreys *et al.* 1967) we attempted to obtain favourable blood gas levels by using 100 % O_2 to ventilate the immature lambs (expts. 508, 509, 430A, 511A, 507, 429B) and 50 % O_2 for the remaining apparently mature lambs; during the last 10 min of ventilation, 100 % O_2 was used for both groups in order to enable us to collapse the lungs subsequently. Ventilation was continued in each case for 2 hr, at which time the following values for blood gas levels (means and ranges) were obtained: for the immature lambs listed above: P_{a,O_2} (mm Hg) = 133 (30–270); P_{a,CO_2} (mm Hg) = 53 (20–100); pH_a = 7·22 (6·90–7·42); and for the remaining lambs: P_{a,O_2} (mm Hg) = 72 (50–120); P_{a,CO_2} (mm Hg) = 32 (30–36); pH_a = 7·38 (7·23–7·44).

Post-ventilation sample of alveolar liquid. Surface tension of lung extracts. In two experiments after killing the lamb at the end of 2 hr ventilation the lungs were excised and placed in a vacuum jar. Evacuation of the jar caused the lungs to collapse, after which liquid could be withdrawn through the trachea. In the remaining

experiments, following a period of ventilation with 100% O_2, the trachea was occluded while the heart continued to beat for a further 5–10 min, when, due to O_2 uptake, the lungs became virtually gas-free and a sample of liquid could then be withdrawn through the trachea. The maximum volume of liquid which could be withdrawn by gentle aspiration was recorded. The minimum surface tension (γ_{min}) obtained on compression of the surface film derived from lung extracts of the upper lobes was measured in an Adam–Langmuir trough as detailed in Humphreys *et al.* (1967).

Measurements of protein concentration and radioactivity

The proteins and [^{125}I]PVP (which contains a mixture of different molecular sizes) in samples of alveolar liquid, lymph and plasma were fractionated by gel filtration on columns of Sephadex G-200 (Pharmacia) as described by Boyd *et al.* (1969) and the K_{av} of the fractions determined, from which the mean radius of equivalent sphere (Einstein, 1905; Sutherland, 1905) of molecules in the fraction was obtained by using the calibration curve in Boyd *et al.* (1969). Protein concentrations and [^{125}I]PVP count rates in both fractionated and unfractionated samples were measured as in Boyd *et al.* (1969).

RESULTS

Transfer of [^{125}I]PVP and protein between alveolar liquid and lymph

Fig. 1 shows results from an illustrative experiment in which [^{125}I]PVP was introduced into alveolar liquid (A_1) and then after a period of control observations lasting 1 hr, positive pressure ventilation was started. In the control period, before starting ventilation, only a negligible amount of [^{125}I]PVP was transferred from alveolar liquid to lung lymph (L_{ly}) or to plasma. With the onset of ventilation (as previously observed by Humphreys *et al.* 1967) an increase in lung lymph flow and a decrease in lymph protein concentration took place, and coincidentally [^{125}I]PVP appeared in lung lymph, the count rate rising at first steeply, but then tending to a plateau at 2 hr after starting ventilation. From the start of ventilation a small amount of [^{125}I]PVP also appeared in plasma. In a sample of alveolar liquid taken at the end of the ventilation period the concentration of [^{125}I]PVP was found to have decreased by about 25% and the protein concentration to have increased from the very low preventilation level of 0·03 g/100 ml. to a value of 1·15 g/100 ml., which is very close to the protein concentration in the last sample of lung lymph collected.

In the thirteen experiments, in which [^{125}I]PVP was put in alveolar liquid, differences between alveolar liquid and lymph were expressed as total concentration ratios; L_{ly}/A_1 for [^{125}I]PVP, where transfer took place from alveoli to interstitial space and hence to lymph, and A_1/L_{ly} for protein where transfer in the opposite direction can be inferred. Two hours after starting ventilation the mean [^{125}I]PVP L_{ly}/A_1 ratio had increased from the preventilation value of 0·00017 (range $0{\cdot}2 \times 10^{-4}$–0·001) to 0·48

($\pm$ 0·09 S.E. of mean); and the protein A_l/L_{ly} ratio from 0·014 (range 0·004–0·037) to 0·70 ($\pm$ 0·08). In the thirteen experiments the ratios for [^{125}I]PVP after ventilation were significantly correlated with the ratios for protein (r = 0·86; n = 13; P < 0·001). There was a wide range in the ratios obtained in different experiments (Table 1).

Volume of alveolar liquid. Since in the foetus prior to the start of venti-

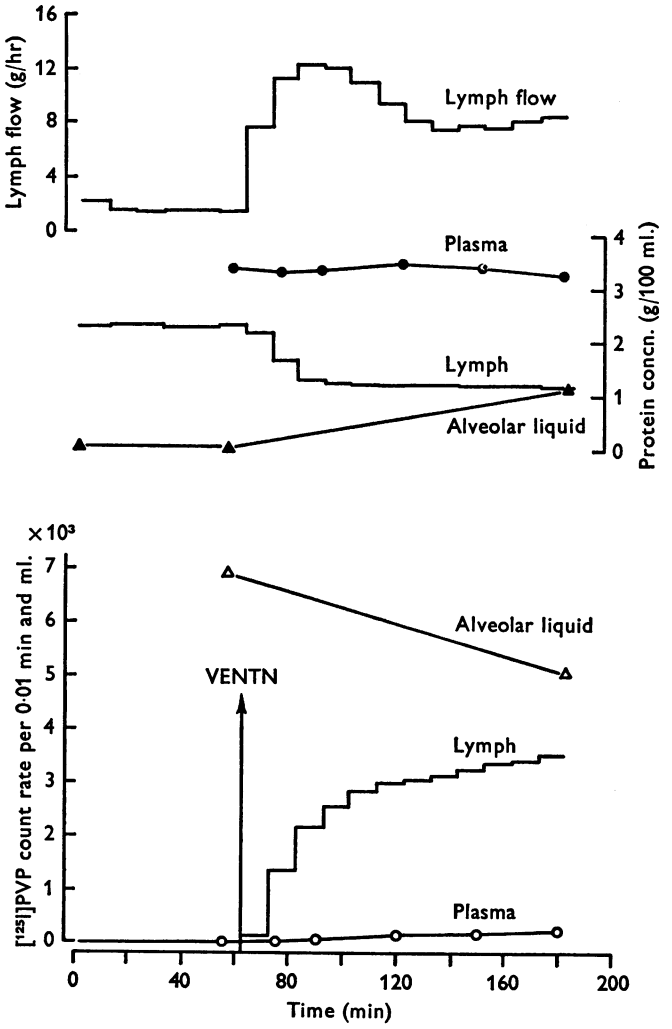

Fig. 1. Expt. 531. Results for lymph flow; protein concentration in plasma, lung lymph and alveolar liquid; and for [^{125}I]PVP counts in alveolar liquid, lung lymph and plasma. At zero time 36 μc [^{125}I]PVP in 3 mg PVP and 0·3 ml. 0·9 % NaCl was mixed with the alveolar liquid. Positive pressure ventilation (VENTN) was started at arrow.

lation practically none of the [^{125}I]PVP injected through the trachea left the interior of the lung, we can estimate the volume of alveolar liquid (V_{al}) from the dilution of the injected radioactivity:

$$V_{al} = V_i . C_i / C_t$$

(where V_i = volume of injected liquid; C_i = counts/ml. and min in injected liquid; C_t = counts/ml. and min in liquid withdrawn after one hour and before starting ventilation).

In thirteen experiments we obtained the following result: mean V_{al} = 23 ml./kg body weight (± 1·6, S.E. of mean), a value similar to the mean difference in lung weight of 27 g/kg between mature foetal and spontaneously delivered new-born lambs more than 6 hr old found by Humphreys *et al.* (1967).

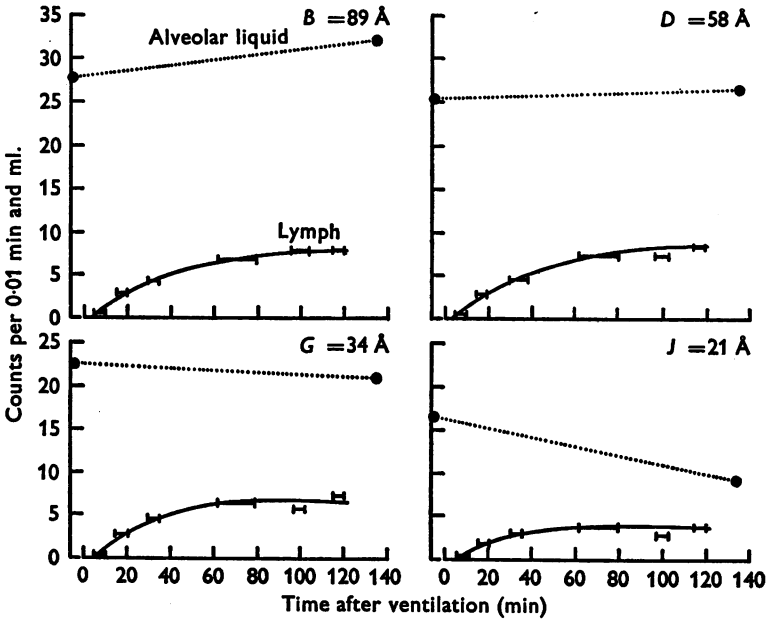

Fig. 2. Expt. 433. Ordinate: count rates (per ml. and 0·01 min) for four PVP fractions in alveolar liquid (●) and lung lymph (horizontal bars). Abscissae: time after onset of ventilation. [^{125}I]PVP had previously been introduced into the alveolar liquid. Smoothed lines have been drawn by eye through the lymph count rates. Mean molecular radius of fractions: B = 89 Å; D = 58 Å; G = 34 Å; J = 21 Å.

Transfer of [^{125}I]PVP from lymph to alveolar liquid

In six experiments on foetal lambs in which [^{125}I]PVP was injected I.V., transfer from plasma to lymph took place readily as described by Boyd *et al.* (1969), but in the foetal state very little transfer to alveolar liquid

could be detected; so that 2·0–3·5 hr after injection the mean [^{125}I]PVP A_l/L_{ly} ratio was 0·008 (range 0·0007–0·013). The immature lamb and one of the mature lambs were ventilated and after 2 hr the [^{125}I]PVP A_l/L_{ly} ratio had increased to 0·98 in the immature and to 0·35 in the mature.

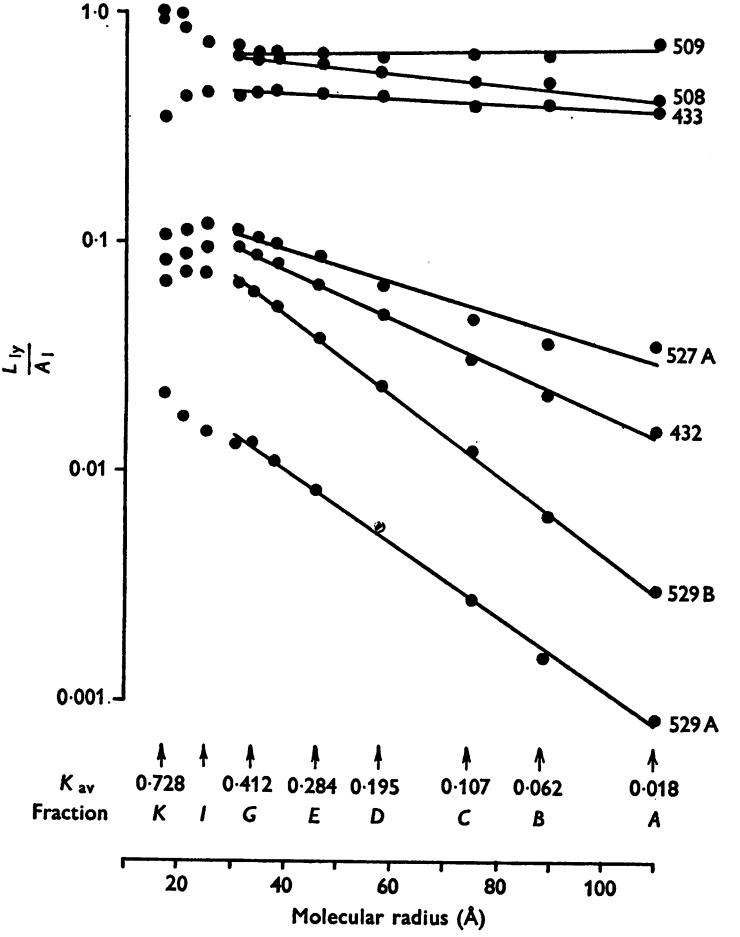

Fig. 3. Lung lymph/alveolar liquid (L_{ly}/A_l) ratios of [^{125}I]PVP gel filtration fractions 2 hr after the start of ventilation. Abscissae: radius of equivalent sphere (molecular radius) with position and K_{av} of fractions *A* to *K*. The scale of the ordinate is logarithmic. Regression lines of log L_{ly}/A_l on molecular radius for fractions *H* to *A* have been fitted by least squares. The slopes of these lines, when multiplied by -10^3, have been termed the Relative Sieving Index (RSI). Values for seven animals are shown; results obtained in the six others were consistent with these but have been omitted to avoid crowding in the upper part of the diagram.

[^{125}I]PVP L_{ly}/A_1 ratios related to molecular size

In the thirteen experiments in which [^{125}I]PVP was put in alveolar liquid, samples of lung lymph collected at intervals after starting ventilation and of alveolar liquid collected before and after a 2 hr period of ventilation were separated by gel filtration into fractions differing in K_{av} and mean molecular radius (radius of equivalent sphere), attention being directed to the eleven fractions *A* to *K* defined by Boyd *et al.* (1969) (K_{av} range 0·018–0·728; molecular radius 110–17 Å). Fig. 2 illustrates the increase in lung lymph [^{125}I]PVP concentration with time after starting ventilation of four of these fractions (*B*, *D*, *G* and *J*) and shows that a fairly steady plateau of concentration was achieved by 2 hr. We decided to compare the transfer of the fractions of different molecular radius in terms of their L_{ly}/A_1 ratio at the end of the 2 hr period of ventilation; Fig. 3 shows values of this ratio for the eleven fractions of different molecular radius in seven of the thirteen experiments. In each experiment (except 509) there was a steady decrease in L_{ly}/A_1 with increasing mean molecular radius between 31 and 110 Å (fractions *H* to *A*), but for fractions with molecular radii between 25 and 17 Å (fractions *I* to *K*) the relationship tended to a plateau. The decrease in ratio from fraction *H* to fraction *A* we attributed to molecular sieving produced by restriction to the passage of [^{125}I]PVP molecules through small openings in alveolar walls; and the plateau in the concentrations of the smaller molecule fractions (*I* to *K*) to their preferential absorption from interstitial space to plasma, for which evidence is given in the last section of Results.

In each of the thirteen experiments, as shown for the seven examples in Fig. 3, regression lines of log L_{ly}/A_1 on molecular radius were fitted by least squares to the values for fractions *H* to *A*. The slopes of the regressions express the proportional decrease in L_{ly}/A_1 ratio per unit of increase in molecular radius. We have used this slope (multiplied by -10^3), which we termed the Relative Sieving Index (RSI), as an empirical expression of the degree to which transfer of a given [^{125}I]PVP fraction is dependent on its mean molecular radius. The higher the value of RSI the greater the sieving and by implication the smaller the openings in alveolar walls. RSI ranged from 17·3 in experiment 529B, in which the greatest degree of sieving occurred, to −0·2 in experiment 509 in which no sieving effect was detectable. Values for RSI as well as for total protein and [^{125}I]PVP ratios are given in Table 1. RSI is significantly correlated with total protein A_1/L_{ly} ($r = -0{\cdot}66$; $n = 13$; $P < 0{\cdot}02$) and with total [^{125}I]PVP L_{ly}/A_1 ($r = -0{\cdot}86$; $n = 13$; $P < 0{\cdot}001$), i.e. the less the total amount of macromolecule transferred the greater the degree of sieving.

TABLE 1. Results from experiments, in which [^{125}I]PVP was put in alveolar liquid, for body weight, gestation, upper lobe minimum surface tension (γ_{min}), peak inflation pressure at 2 hr, terminal liquid volume and post-ventilation values for protein ratio (A_l/L_{ly}) and [^{125}I]PVP ratio (L_{ly}/A_l) and Relative Sieving Index (RSI)

Expt. no.	Body wt. (kg)	Gestational age (days)	γ_{min} (dyn/cm)	Peak inflation pressure (cm H_2O)	Terminal liquid volume (ml./kg)	Protein $\frac{A_l}{L_{ly}}$	[^{125}I]PVP $\frac{L_{ly}}{A_l}$	RSI
529A	4·6	138	—	20	0·16	0·31	0·010	15·9
529B	4·5	138	1	20	0·04	0·68	0·036	17·3
432	6·2	—	4	—	0·32	0·23	0·040	10·5
527A	3·5	140	7	28	0·14	0·33	0·078	7·1
433	6·0	—	7	—	3·0	0·67	0·42	1·1
508	3·8	128	18	45	4·0	0·94	0·53	2·4
509	3·6	130	12	44	0·85	0·91	0·59	−0·2
430A	3·4	—	11	—	3·8	0·71	0·63	1·5
531	4·8	141	14	33	1·7	1·01	0·70	1·8
511A	3·5	128	18	45	0·57	0·70	0·74	0·6
528	4·6	137	10*	37	5·2	1·04	0·79	1·8
507	3·9	126	16	35	1·3	0·98	0·82	1·0
429B	2·5	—	12	—	2·4	1·01	0·88	1·6

* Lower lobe value.

TABLE 2. Correlations of γ_{min}, peak inflation pressure and terminal liquid volume with post-ventilation ratios for protein (A_l/L_{ly}) and [^{125}I]PVP (L_{ly}/A_l) and with Relative Sieving Index (RSI)

		Protein $\frac{A_l}{L_{ly}}$		[^{125}I]PVP $\frac{L_{ly}}{A_l}$		RSI	
	n	r	P	r	P	r	P
γ_{min}	12	0·60	$0·05 > P > 0·025$	0·78	$0·005 > P > 0·001$	−0·78	$0·005 > P > 0·001$
Peak inflation pressure	9	0·59	$> 0·10$	0·77	$0·02 > P > 0·01$	−0·90	0·001
Terminal liquid volume	13	0·59	$0·05 > P > 0·025$	0·57	$0·05 > P > 0·025$	−0·55	0·05

Transfer of protein and [^{125}I]PVP between alveolar liquid and lymph related to maturity of lung

Table 1 gives data for body weight, gestational age, and for three measurements which we accepted as being related to lung maturity (cf. Humphreys *et al.* 1967); i.e. minimum surface tension (γ_{min}) of upper lobe lung extracts, the peak inflation pressure recorded near the end of the

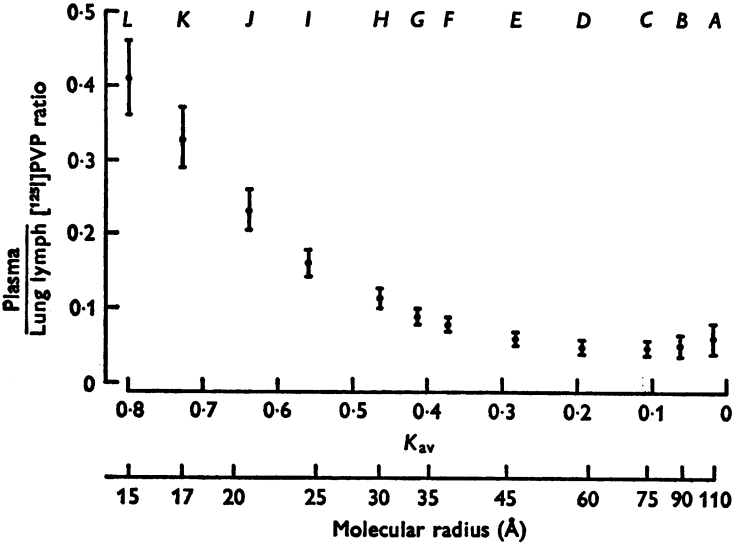

Fig. 4. Mean plasma/lung lymph ratios (S.E. of mean shown as vertical bar; $n = 13$) for twelve [^{125}I]PVP gel filtration fractions *A* to *L*. Abscissae: K_{av} and molecular radius (Å). [^{125}I]PVP had been put in alveolar liquid. Samples collected after 2 hr of ventilation.

2 hr period of positive pressure ventilation and the volume of liquid (per kg body weight) which could be aspirated after ventilation (terminal liquid volume). There was no significant correlation in the range examined, between either gestational age or body weight on the one hand and RSI or the post-ventilation ratios of total protein or [^{125}I]PVP on the other. As shown in Table 2, significant positive correlations were found between the post-ventilation [^{125}I]PVP ratios and each of the measurements related to lung maturity (γ_{min}, peak inflating pressure at 2 hr, and terminal liquid volume); and between post-ventilation protein ratios and two of these measurements (γ_{min} and terminal liquid volume). Significant correlations were found between RSI and all three of the factors. These correlations show that when immature foetal lungs are first ventilated with positive pressure, the total amount of macro-molecular transfer between alveoli

and lymph is greater, and that the degree of molecular sieving is less, than when mature lungs are ventilated.

Transfer of [^{125}I]PVP from lymph to plasma

A small but significant increase in plasma [^{125}I]PVP counts, similar to that shown in Fig. 1, was observed after the onset of ventilation in each experiment, in which [^{125}I]PVP had been put in alveolar liquid. [^{125}I]PVP could have entered plasma by transfer across lung capillary walls as well as by drainage via the right lymph duct, which was left intact in the dissection, and which in the foetal lamb drains about a third of the pulmonary lymph (Humphreys *et al.* 1967). Fig. 4 gives values for plasma/lung lymph ratios of the [^{125}I]PVP fractions obtained by gel filtration of lymph and plasma samples collected after 2 hr ventilation; (twelve fractions *A* to *L* are shown in this case, which extends the range in molecular radius down to 15 Å). For the range of fractions *A* to *G* (molecular radii 110–34 Å), over which sieving of [^{125}I]PVP in transfer from plasma to lymph was demonstrated by Boyd *et al.* (1969), the ratios do not differ significantly, suggesting that the transfer of these fractions to plasma depends on drainage through the intact right lymph duct system rather than on transfer across capillary walls. For fractions *G* to *L* (molecular radii 34–15 Å) there was a systematic increase in ratio with decreasing molecular radius, which suggests that substantial amounts of these molecules were absorbed directly into lung capillaries, their rates of transfer depending on molecular size, presumably because of the restriction imposed in passing through pores in capillary walls.

DISCUSSION

Transfer of macromolecules between alveolar and interstitial spaces in the foetus

In the foetal state so little [^{125}I]PVP crossed from alveolar liquid to lymph or in the reverse direction that we can infer the presence of a virtually complete barrier between alveolar and interstitial spaces to this mixture of macromolecules (i.e. down to a Stokes–Einstein radius of less than 17 Å). The presence of an alveolar–interstitial barrier to macromolecules probably also explains the very low concentration of protein in alveolar liquid as compared with lymph.

The possibility had to be considered that incomplete diffusional mixing of the [^{125}I]PVP in small liquid-filled lung units was the reason for the lack of transfer from alveolar liquid to lymph; but we considered that incomplete mixing was unlikely because West, Dollery, Matthews & Zardini (1965) had shown in the liquid-filled lung that [^{131}I]albumin introduced

through the trachea in a tidal volume 25% that of the total lung became evenly mixed between 9 and 30 min after a single injection, and in our experiments we used a larger injection volume (50% total lung volume) which was withdrawn and re-injected four times, and we waited for 1 hr after introducing the [^{125}I]PVP before starting ventilation. The absence of transfer to alveolar liquid of intravenously injected [^{125}I]PVP, which undoubtedly became well mixed with plasma and lymph, confirmed that the barrier existed at the alveolar wall.

There is in the foetal lamb a striking contrast between the impermeability of the barrier between alveoli and interstitium (which must correspond with the layer of alveolar epithelial cells) and the relatively much greater permeability to macromolecules of lung capillary walls, which is sufficient to maintain, in the steady state, a lymph/plasma ratio of 0·5 for macromolecules of Stokes–Einstein radius $\approx$ 55 Å (Boyd *et al.* 1969). Similarly, in liquid-filled isolated perfused lungs from the rabbit, Wangensteen, Wittmers & Johnson (1969) found a much smaller permeability constant for the transfer of sucrose across alveolar walls than for its transfer across capillary walls; and, in electron micrographs, Schneeberger-Keeley & Karnovsky (1968) have shown that horseradish peroxidase used as a tracer passes readily through pores between lung capillary endothelial cells, but that the layer of alveolar epithelial cells is impermeable to this substance. These findings imply that the impermeability of the alveolar–interstitial barrier to macromolecules, which we have shown here in the foetal lamb, is probably a characteristic of lungs in general, and persists into the free-living air-breathing period of life.

Break-down of alveolar–capillary barrier produced by ventilation

It seems likely that in our experiments the movements of ventilation caused breaks in the alveolar cell layer, through which macromolecules could pass. There were large differences between experiments in the total amount of [^{125}I]PVP and protein transferred; and the values of RSI, which ranged from effectively zero ($-0{\cdot}2$) to 17·3 and were significantly correlated with the post-ventilation [^{125}I]PVP ratios, indicate that there was a corresponding variation in the size of the breaks (i.e. the larger the breaks the more the total amount of molecular transfer).

We can make a rough comparison between the size of the breaks produced by ventilation in alveolar walls and the size of the pores in lung capillary walls. From data in Boyd *et al.* (1969) we can calculate a value of RSI = 7·3 for lung capillary walls in mature foetal lambs, which we can compare with our RSI values for alveolar walls after ventilation. We can conclude that, in expts. 529 A, 529 B and 432, the degree of molecular sieving caused in crossing alveolar walls was greater than that caused in

crossing lung capillary walls; and that in these experiments the average radius of the breaks produced in the alveolar walls by ventilation is likely to have been less than the value of 150 Å, which Boyd *et al.* (1969) assigned to lung capillaries in mature foetal lambs. In the remaining experiments the breaks must have been larger; and in expt. 509, where there was no detectable sieving, we can calculate (from eqn. 7–10 in Landis & Pappenheimer, 1963) that the breaks are likely to have been larger than 1000 Å in radius.

Effect of lung maturity and other factors on break-down of alveolar–interstitial barrier

It seems inherently likely that the less the degree of break-down in alveolar–interstitial barrier the nearer we approach the behaviour of the normal mature lung at the start of breathing; and although the differences between animals in this respect were not shown to be significantly correlated with gestational age, for which we had incomplete and possibly inaccurate data, we did obtain evidence that the more mature the lung the less the degree of break-down after 2 hr ventilation. The susceptibility of the less mature lungs to disruption of the alveolar–interstitial barrier is of particular interest as an explanation for the leakage of protein-rich liquid into air spaces leading to the formation of hyaline membranes, which are characteristic of ventilated immature lungs but never seen in foetal lungs (Gitlin & Craig, 1956; Gajl-Peczalska, 1964). However, as we found it necessary to ventilate the immature lungs with higher pressures and at higher O_2 concentrations than the mature lungs, we cannot be sure that the differences we incline to attribute to immaturity are not in fact attributable to one or other of these factors, or to the asphyxia which cannot always be avoided when external gas exchange depends on immature lungs (see blood gas measurements for immature lambs in Methods).

This work was supported by a grant from the Medical Research Council. I.C.S.N. was supported by a grant from the Association for the Aid of Crippled Children, New York; E.O.R.R. by a grant from the Wellcome Trust. The technical help of Mr C. M. J. Bright and Miss V. Cole is gratefully acknowledged.

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